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The Genotype of Human Papillomavirus and Associated Factors Among High Risk Males in Shanghai, China: A Molecular Epidemiology Study

Authors' Contribution:
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Statistical Analysis C
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Manuscript Preparation E
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Background: The aim of this study was to describe the infection rate of human papillomavirus (HPV), its genotype distribution, and associated factors among high-risk males in Shanghai, China.

Material/Methods: A total of 1,205 participants were enrolled in this study from October 2015 to March 2017. Each individual signed an informed consent before agreeing to take part in this study; participants completed a questionnaire about associated factors related to HPV infection. The HPV GenoArray test kit was used to perform HPV genotyping and was also used in DNA amplification and HybriBio's proprietary flow-through hybridization technique.

Results: The overall infection rate of HPV was 58.4%. HPV 11 (177, 14%) was the most common low-risk (LR) genotype and HPV 16 (78, 6.47%) was the top prevalent high-risk (HR) genotype among all the male patients. Additionally, multiple infections (387, 32.1%) was the most common type among all the infected cases, while single infection was only 317 cases (26.3%). Sexual preference, number of sex partners, sexual activity, and frequency of condom use were significantly associated with HR and LR infection.

Conclusions: The infection rate of HPV infection among high-risk males in Shanghai was very high.

MeSH Keywords: **China • Genotype • Human Papillomavirus DNA Tests**

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Background

Human papillomavirus (HPV) infection is an important pathogenic factor of condyloma acuminatum (CA), which is one of the most common sexually transmitted diseases (STD), and affects as many as 30 million individuals [1]. More than 100 subtypes of HPV have been reported [2]. Recently, an increasing number of studies have demonstrated the close relationship between cancer of the anus and penis and long-term infection of HPV, especially in men who have sex with men (MSM) [3–6]. The prevalence of CA in the anus and penis has increased as has the practice of receptive and anal intercourse among MSM. Much has been learned about HPV prevalence in women [7–9], so it is important to investigate the prevalence and genotype of HPV among high-risk males and the associated risk factors.

To the best of our knowledge, no research has reported on the prevalence and genotype distribution of HPV among males in Shanghai. However, the population composition of Shanghai is relative complex because of the large number of transient people in the population. Therefore, the objective of our study was to describe the prevalence and distribution of HPV genotype among high-risk males in Shanghai and discuss the potential risk factors.

Material and Methods

Study population

A total of 1,205 male patients were enrolled in this study from October 2015 to February 2017. All the participants were recruited from the Dermatology Department of Shanghai Songjiang Jiuting Hospital and Shanghai General Hospital. Each individual signed an informed consent before agreeing to take part in this study and completed a questionnaire about associated risk factors. All the male patients had been involved in sexual activity recently. The participants were excluded if they once received an HPV vaccine or reported a serious illness. This study was granted permission by Shanghai Songjiang Jiuting Hospital and Shanghai General Hospital Institutional Review Board.

Sample collection

For all the study patients, a pre-wetted saline iCleanhcy flocced swab was used to collect specimens from the penis, glans penis or coronary sulcus by a dermatologist. For each patient, each sample was placed into a separate vial containing 1 mL of PreservCyt solution and the tubes that contained the specimens were stored at room temperature. A sample was collected independently for HPV genotyping assay.

HPV DNA detection

For HPV DNA extraction, all the samples were stored in a specimen transport medium and sent to the laboratory of our hospital. High-quality DNA was obtained from disruption of the cells; DNA isolation and purification was performed according to the manufacturer's instructions for DNA extraction. HPV detection and genotyping were performed using the HPV Genotyping Kit (HybriBio Ltd.), a PCR-based flow-through hybridization and gene chip system. All sequencing primers and sequencing steps were based on previous descriptions. In all, 26 type-specific probes which recognize 17 HR (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68, and 73) and nine LR HPV genotypes (6, 11, 40, 42, 43, 44, 54, 55, and 57) were contained in the gene chip. According to the manufacturer's protocols, the PCR reaction was used to amplify the extracted DNA. The final results were obtained by colorimetric change on the chip under direct visualization. HPV negative and positive controls provided in the kit were simultaneously detected in every test.

Statistical analysis

All statistical analyses were performed with a statistical software package (SAS version 9.0; SAS Software, Cary, USA). The prevalence of HPV infection, genotype distribution, single and multiple HPV infections were analyzed separately. To explore the associations of several risk factors with HPV infection, a logistic analysis was performed. The 95% confidence intervals (CIs) for prevalence were calculated by the binomial exact test.

Results

Genotypes distribution of HPV

Study patient age range was 16–67 years, median 33 years. A total of 1,205 samples were collected and tested for HPV genotypes. The genotype test showed 704 samples were HPV positive; the overall infection rate of HPV was 58.4%. Among all the infected male patients, 482 patients (68.5%) had CA of the penis, 187 patients (26.6%) had CA of the anus or anal canal, 35 patients (4.9%) had all CA sites infected. The participants were divided into four age groups: ≤ 20 years of age, 21–35 years of age, 36–50 years of age, and ≥ 51 years of age. The total infection rate, HR infection rate, and LR infection rate are shown in Figure 1.

The details of genotypes distribution of all the HPVs were as follows: HPV11 (177, 14.69%), HPV 6 (156, 12.95%), HPV 16 (78, 6.47%), HPV 58 (65, 5.39%), HPV 43 (54, 4.48%), HPV 18 (48, 3.98%), HPV 52 (30, 2.49%), HPV 67 (24, 1.99%), HPV 51 (22, 1.83%), HPV 33 (20, 1.66%), HPV 56 (19, 1.58%), HPV 54

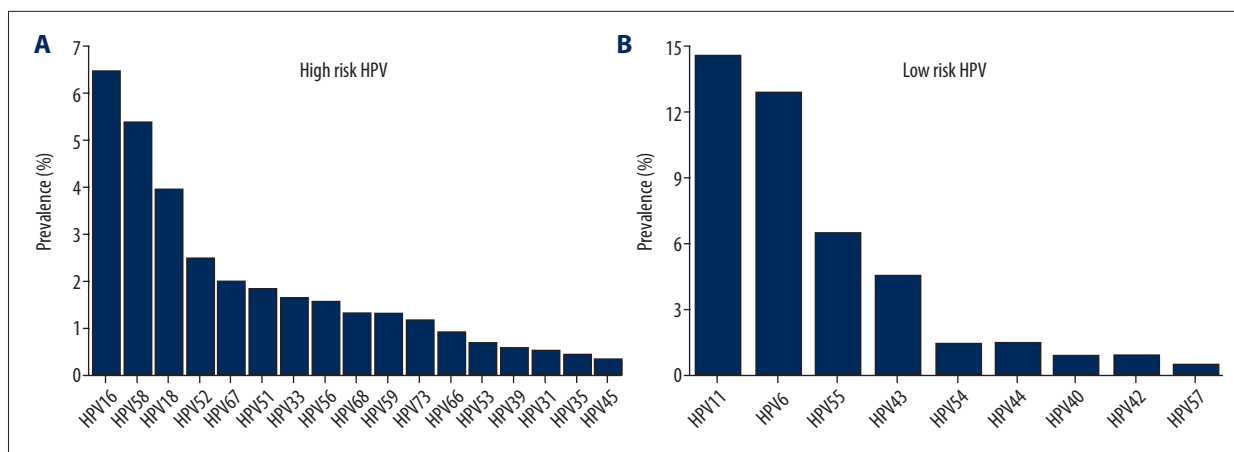


Figure 1. Distribution of HPV genotypes among sample: (A) infection rate of HR HPV among samples; (B) infection rate of LR HPV among samples.

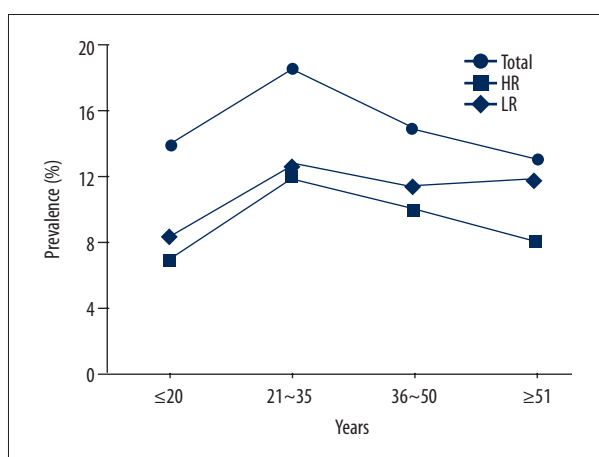


Figure 2. Infection rate and of HPV among samples grouped by age.

(17, 1.41%), HPV 68 (16, 1.33%), HPV 59 (16, 1.32%). HPV 73 (14, 1.16%), HPV 55 (12, 1.00%), HPV 66 (11, 0.91%), HPV 40 (10, 0.83%), HPV 42 (10, 0.83%), HPV 53 (8, 0.66%), HPV 39 (7, 0.58%), HPV 31 (6, 0.50%). HPV 44 (6, 0.50%), HPV 35 (5, 0.41%), HPV 57 (5, 0.41%), and HPV 45 (4, 0.33%). Notably, HPV 11 (177, 14%) was the most common LR genotypes and HPV 16 (78, 6.47%) was the top prevalent HR genotypes among all the male patients. The prevalence of HR and LR genotypes are displayed in Figure 2.

Prevalence of single and multiple infections

The results show cases of multiple infections (387, 32.1%) was the most common type among all the infected cases while cases of single infection was only 317 (26.3%). Moreover, the prevalence decreased obviously with the increased number of infection genotypes among multiple infections, with 221 patients (18.3%) with double infection, 112 patients (9.3%) with triple infection, 38 patients (3.2%) with quadruple infection,

nine patients (0.7%) with quintet infection, five patients (0.4%) with sextuple infection, and two patients (0.2%) with octuple infection.

Sociodemographic and characteristics of the participants and risk factors

Table 1 shows the sociodemographic and characteristics of the participants in this study. Most of the participants had less than 12 years of education (58.9%) and an income of more than 3,000 RMB every month (65.6%). Furthermore, the majority of the samples were from: heterosexual males (57.9%), married males (59.1%), had at least two sexual partners (51.2%), used condom (76.1%), had no STDs diagnosis (87.7%), and were smokers (74.4%). In addition, we conducted univariate analyses of all risk factors and found all the risk factors were related with infection ($p < 0.05$).

Table 2 displays the associated factors for HR and LR infection on the multivariate logistic regression model. We found sexual preference, number of sex partners, sexual activity, and frequency of condom use were significantly associated with HR and LR infection ($p < 0.05$). Additionally, Table 3 displays the associated factors for single and multiple infections on the univariate and multivariate logistic regression model. We found sexual preference, marital status, number of sex partners, sexual activity, and frequency of condom use were significantly associated with single and multiple infections ($p < 0.05$).

Discussion

CA, caused by various kinds of HPV, infects a broad range of the population worldwide, despite the undetermined exact frequency [10,11]. Therefore, research into the prevalence and genotype distribution of HPV in different areas and periods

Table 1. Sociodemographic and characteristics of the participants in this study.

Factor	n (%)	n		p Value
		Infected	Not infected	
Total	1205	704	501	
Age (years)				
20 or less	211 (17.5)	163	48	0.00
21 to 35	432 (35.9)	217	215	
36 to 50	306 (25.4)	181	125	
≥51	256 (21.2)	143	113	
Education (years)				
<12	710 (58.9)	289	421	0.00
≥12	495 (41.1)	415	80	
Monthly income (in RMB)				
<3000	415 (34.4)	120	295	0.00
3001 to 5000	489 (40.6)	349	140	
>5000	301 (25.0)	235	66	
Sexual preference				
Homosexual	380 (31.5)	271	109	0.00
Heterosexual	698 (57.9)	321	377	
Bisexual	127 (10.5)	112	15	
Marital status				
Married	712 (59.1)	471	241	0.00
Single/Divorced	493 (40.9)	233	260	
Number of sex partners				
1	588 (48.8)	176	412	0.00
2	371 (30.8)	248	123	
≥3	246 (20.4)	230	16	
Sexual activity				
Vaginal penetration	312 (25.9)	116	196	0.00
Anal penetration	432 (35.9)	210	222	
Any genital contact	461 (38.3)	358	103	
Frequency of condom use				
Every time	156 (12.9)	40	106	0.00
Sometimes	761 (63.2)	420	341	
Never	288 (23.9)	244	44	
Previous STDs diagnosis				
Yes	148 (12.3)	125	23	0.00
No	1057 (87.7)	579	478	
Smoking status				
Yes	897 (74.4)	541	356	0.02
No	308 (25.6)	163	145	

STD – sexually transmitted disease.

Table 2. Multivariate analyses of risk factors with HR and LR infection.

Factor	HR infection		LR infection	
	AOR (95% CI)	p Value	AOR (95% CI)	p Value
Sexual preference				
Homosexual	1.0		1.0	
Heterosexual	1.1 (0.4, 2.0)	0.02	0.8 (0.5, 1.7)	0.03
Bisexual	1.5 (1.1, 3.2)		1.3 (1.0, 3.0)	
Number of sex partners				
1	1.0		1.0	
2	1.9 (0.9, 3.4)	0.00	1.7 (0.9, 3.2)	0.00
≥3	2.4 (1.2, 6.2)		2.4 (1.2, 6.6)	
Sexual activity				
Vaginal penetration	1.0		1.0	
Anal penetration	1.3 (0.7, 3.4)	0.02	1.8 (0.9, 3.8)	0.01
Any genital contact	2.5 (1.2, 6.9)		3.6 (1.6, 8.5)	
Frequency of condom use				
Every time	1.0		1.0	
Sometimes	1.8 (0.6, 3.5)	0.01	1.7 (0.9, 3.5)	0.00
Never	2.2 (1.2, 6.0)		2.9 (1.3, 8.0)	

Factors that were considered but were not significant are not listed in this table, including age, education, monthly income, marital status, previous STDs diagnosis, smoking status. AOR – adjusted odds ratios; CI – confidence interval; HR – high risk; LR – low risk; STD – sexually transmitted disease.

is important. Because of the growing percentage of MSM and open sexual behavior, we focus on the prevalence and genotype distribution of HPV among high-risk males in Shanghai in this study.

In our study, the overall infection rate of HPV among males was 58.4%, which was higher than many other studies [12,13]. Furthermore, we assessed 26 genotypes of HPV, which include 17 HR and nine LR HPV genotypes with the prevalence of 32.6% for HR types and 43.5% for LR types among male study patients, which was much higher than the Henan et al. study [14] which reported 6.4% for HR types; and the Guangxi et al. [15] study which reported 9.4% for HR types. This could be explained by different anatomic sampling sites and different HPV typing techniques, as well as, perhaps most important, the relative well-developed area of Shanghai with more open sexual behavior attitudes that may led to more chances of infection by HPV.

Information about the age-specific prevalence of HPV infection is also important for designing vaccination programs for patients for different age groups. We observed a peak of prevalence around the age of 21 to 35 years in HR, LR and total infection, which could be caused by more sexual activity

induced by hormonal levels of males during this age period. Although the distribution of HPV genotypes, especial HR genotypes, shows diversity in different areas, HPV 16 was still the top prevalent HR genotypes among all the male patients in our study, which was consistent with research from Brazil, America and Anyang, China [8,16]. However, other studies have reported different results in terms of the most common HR HPV genotypes, such as Liuzhou, China (HPV52) and Mexico (HPV59) [17]. Moreover, HPV 11 and HPV 6, the most common two genotypes in our study and other studies, which are the etiologic agents of over 90% of CAs (including location in the anus and penis) and could be explained by the increasing percentage of MSM among males [18,19]. Our results of the study questionnaires also favored this phenomenon: 42% of the male participants reported homosexual behavior and 35.9% reported anal penetration (Table 1). The data in Table 2 also demonstrates that anal penetration and any genital contact are risk factors which lead to HPV infection.

Several risk factors associated with HPV infection show differences by country of residence [16,20,21]. It was expected that increasing number of sexual partners, anal penetration, and any genital contact, and never using a condom would be

Table 3. Multivariate analyses of risk factors with single and multiple infection.

Factor	Single infection		Multiple infection	
	AOR (95%CI)	p Value	AOR (95%CI)	p Value
Sexual preference				
Homosexual	1.0		1.0	
Heterosexual	1.2 (0.6, 3.0)	0.02	0.9 (0.5, 2.0)	0.00
Bisexual	1.7 (0.9, 5.0)		2.6 (1.5, 5.9)	
Marital status				
Married	1.0		1.0	
Single/Divorced	0.5 (0.1, 1.1)	0.01	1.5 (0.5, 2.2)	0.00
Number of sex partners				
1	1.0		1.0	
2	1.7 (1.1, 2.6)	0.00	2.7 (1.5, 7.5)	0.00
≥3	2.6 (1.5, 7.4)		5.1 (2.5, 11.9)	
Sexual activity				
Vaginal penetration	1.0		1.0	
Anal penetration	1.7 (0.7, 2.8)	0.01	2.7 (1.2, 8.5)	0.00
Any genital contact	2.4 (1.2, 6.8)		4.3 (2.7, 10.0)	
Frequency of condom use				
Every time	1.0		1.0	
Sometimes	1.5 (0.5, 3.1)	0.01	2.1 (1.1, 5.4)	0.00
Never	2.3 (1.2, 6.6)		3.1 (2.2, 9.5)	

Factors that were considered but were not significant are not listed in this table, including age, education, monthly income, previous STDs diagnosis, smoking status. AOR – adjusted odds ratios; CI – confidence interval; STD – sexually transmitted disease.

associated with more chances of HR and LR HPV infection. Besides these aforementioned factors, being single or divorced also increased the chances of single and multiple infections. It is well established that HR genotypes, especially HPV16, have an increased risk of clinically relevant anogenital lesions development. Heterosexual and bisexual behavior seems to increase the risks of HR HPV infection (Table 2). A likely reason for this phenomenon might be more open attitudes towards sex with the development of economic opportunities and improvements in living standards in Shanghai, China.

There were several limitations to this study. First, we included a relative small number of participants, which may not reflect the overall situation of Shanghai, China. Second, some

potentially important factors related to HPV infection were not included in this study, such as number of sexual activities in the past month, so the relationship between these additional factors should be included in future studies.

Conclusions

This prospective study reports high rate of HPV infection among high-risk males in Shanghai, including HR and LR genotypes, and single and multiple infections. The present study demonstrated several significant risk factors associated with HPV infection.

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